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Full Length Research Paper

Interaction of arbuscular mycorrhizal fungus (*Glomus intraradices* and *Glomus etunicatum*) with tomato plants grown under copper toxicity

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In this research, the effect of two arbuscular mycorrhizal fungal (AMF) inoculation (*Glomus intraradices and Glomus etunicatum*) on tomato plants growing in nutrient solution with high concentrations of copper were studied. Copper (Cu) is an essential micronutrient for plant growth. In the present study, the effect of copper toxicity on growth, chlorophyll, sugar, protein content and antioxidant enzymes activity in both mycorrhizal and non-mycorrhizal tomato plants were studied. The experiment was performed by using two treatments (mycorrhizal and non-mycorrhizal) and five concentrations of $CuSO_4$ solution (0, 1.5, 3.5, 5.5, 7.5 Mm $CuSO_4$) added to Hogland nutrient solution (with half P concentration). Copper toxicity caused reduction in growth in all treatments (non-AM). This may be due to the accumulation of Cu in leaves. Fresh and dry weights of shoot and roots, total sugar content in both shoots and roots, the total chlorophyll content in leaves, protein content in shoots and roots, antioxidant enzyme activity and root colonization were determined.

Key words: Arbuscular mycorrhiza, tomato plants, copper toxicity, *Glomus intraradices, Glomus etunicatum*, oxidative stress.

INTRODUCTION

Soil ecosystems have been extensively contaminated with heavy metals due to various human activities, possibly including mobilization of these metals in the food chain, thereby threatening human health. Providing a direct physical link between soil and plant roots, the arbuscular mycorrhiza (AM) fungi are important rhizospheric microorganisms. They can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003), and consequently they increase root and shoot biomass and improve plant growth. It has been indicated that AM fungi can make colonization with plant roots in metal contaminated soil (Vogel-Mikus et al., 2005), while their effects on metal uptake by plants are conflicting. In slightly metal contaminated soil, most studies show that AM fungi increased shoot uptake of metals (Weissenhorn et al., 1995), while in severely contaminated soil, AM fungi could reduce shoot metal concentration and protect plants against harmful effects of metals (Li and Christie, 2001; Malcova et al., 2003). Both fungal isolates and plants may vary in their individual or combined tolerances to metals. Optimizing the use of AM fungi to permit growth of plants in soils contaminated with metals may require careful selection of specific fungal and host plant combinations for a given set of soil conditions. It will also require a skillful use of inorganic and organic amendments to maximize plant growth and capitalize the interactions or competitions between metals and elements such as phosphorus and sulfur. Host uptake is generally enhanced in mycorrhizal plants. For example, increased P through bioorganic or inorganic phosphate-amendments may increase plant biomass and thus perhaps detoxify the potential effects of metals by dilution, precipitation or adsorption of metals onto

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Abbreviations: AMF, Arbuscular mycorrhizal fungal; **GUPX,** guaiacol peroxidase; **APX,** ascorbate peroxidase; **SOD,** superoxide dismutase; **CAT,** catalaz; **PO,** peroxidase.

polyphosphate granules. The non-target ecological effects of plants or fungi which have adsorbed, translocated and sequestered metals also need to be considered in parallel with the efforts to revegetate soils contaminated with high levels of metals. Efforts to phytoremediate, reclaim or restore vegetation to soils contaminated with metals by use of mycorrhizal plant species and inocula is gaining acceptance (Entry et al., 2002; Khan et al., 2000; Turnau and Mesjasz-Przybylowicz, 2003; Vogel-Mikus et al., 2005).

Interveinal foliar chlorosis is a common initial symptom of Cu toxicity (Taylor and Foy, 1985; Zhu and Alva, 1993). Chlorosis was also displayed in *Banksia ericifolia* (heath banksia), *Casuarina distyla* (she-oak) and *Eucalyptus eximia* (yellow bloodwood) when grown at elevated Cu (Joner et al., 2000). The chlorosis often takes the form of cream or white spots or lesions (Lee et al., 1996; O'Sullivan et al., 1997). With increasing exposure, leaf tips and margins can become necrotic (Taylor and Foy, 1985; Yau et al., 1991). In acute Cu toxicity, leaves may become wilted before eventually becoming necrotic (Yau et al., 1991). Copper toxicity can be associated with a purpling of foliage (Choi et al., 1996), but this is not apparent in all species (O'Sullivan et al., 1997). Copper toxicity has a significant effect on root growth and form, often before any effect on above-ground growth (Minnich et al., 1987). Patterson and Olson (1983) found that the germination of six tree species was less sensitive to Cu than subsequent root elongation. In dicot seedlings, toxic amounts of Cu result in radicles which are short, blunt tipped of dark brown/black colouration (necrotic) and have a disposition to fungal attack (Patterson and Olson, 1983). *Citrus paradisi* × *Poncirus trifoliata* (swingle citrumelo) seedlings exposed to excess Cu produce few new roots and have a thickened tap root (Zhu and Alva, 1993). Thickening of root apicies was also apparent in Pinus seedlings (Arduini et al., 1995). In *Betula papyrifera* (paper birch) and *Lonicera tatarica* (honeysuckle), seedlings high Cu concentrations have been shown to inhibit the production of root hairs (Patterson and Olson, 1983).

Arbuscular mycorrhizal fungi (AMF) have multiple beneficial effects on the growth of plants. The fine hyphae of the fungi effectively mobilize water and nutrients such as phosphorus, nitrogen, potassium, calcium, iron and copper from soil particles, and these nutrients are then transferred to the host plants (Jakobsen et al., 1992; Johansen et al., 1992; Kothari et al., 1991; Smith and Benitez 1955). AMF-colonized plants are generally more resistant to stresses caused by drought, salt, heavy metals or attack by pathogens. These positive effects of the fungi on the growth of plants often result from an improved nutrient supply, and can partly be due to complex and not easily resolved interactions between the symbiotic partners. With regard to heavy metal stress, the literature is somewhat controversial. At low concentrations, several heavy metals such as Zn, Cu, Mn or Mo are micronutrients. Published data indicate that the colonization of roots by AMF results in an enrichment of these metal ions in the low concentration range (Diaz, 1996; Leyval et al., 1997). In contrast, AMF-colonized roots of plants from soils severely polluted by high concentrations of heavy metals show lower amounts of heavy metals than non-colonized plants (El-Kherbawy et al., 1989; (Kaldorf et al., 1999; Schu¨epp et al., 1987; Smith and Benitez 1955), but see also opposite data by Gildon and Tinker (1983). At high heavy metal concentrations, the elements that unavoidably reach the inside of the roots are concentrated in the inner root parenchyma cells, where there are intraradical fungal structures such as arbuscules, vesicles and intraradical hyphae (Kaldorf et al., 1999). This observation is largely in agreement with the findings of an earlier study with AMF-colonized roots of *Pteridium aquilinum* (Turnau and Mesjasz-Przybylowicz, 2003). Although, biophysical methods do not allow discrimination of heavy metal distribution between fungal and plant cell structures, the fungi obviously direct heavy metal allocation within the roots. Several mechanisms for this allocation can be envisaged (Galli et al., 1994; Leyval et al., 1997; Schu¨epp et al., 1987): (a) heavy metals may be bound to the cell wall and may be deposited in the vacuoles of the fungi, (b) heavy metals may be attached to siderophores and sequestered either into the root apoplasm or into the soil, (c) heavy metals may be bound to metallothioneins or phytochelatins inside the fungal or plant cells, or (d) heavy metal transporters at the plasmalemma or tonoplast of both partners may catalyze the export of heavy metals from the cytoplasm. The alterations in heavy metal content in roots upon colonization by the fungi suggest that extensive changes in gene expression occur, presumably both at the transcriptional and the translational levels. Thus, heavy metal tolerance of plants conferred by AMF colonization cannot easily be resolved because of the multiplicity of factors involved.

MATERIALS AND METHODS

Surface sterilized seeds of used tomato (*Lycopersicon esculentum*) were sowed in the sterilized soil /sand mixture. The mycorrhizal inoculum used was stock culture of *Glomus etunicatum* which was bulked in an open pot culture of *Zea mays* L. Mycorrhizal treatments were carried out by adding 20 g per pot of mycorrhizal inoculum from stock culture which was placed below tomato seeds. Non-mycorrhizal treatments received the same quantity of autoclaved inoculum. Plants (three per pots) were grown for 60 days in a growth chamber in Urmia University with temperatures ranging from 19 to 25°C, a 16/8 h light/dark period, and a relative humidity of 70 to 80%. One week after emergence of seedlings, each pot received 50 ml of modified Hoagland´s nutrient solution (mg L^{-1}) with half P concentration three times a week by adding 0 (control), 1.5, 3.5, 5.5, 7.5 mM $CuSO_4$ to the nutrient solution. Plants received 50 ml nutrient solution three times a week for about 91days. The plants were planted in February and harvested in April.

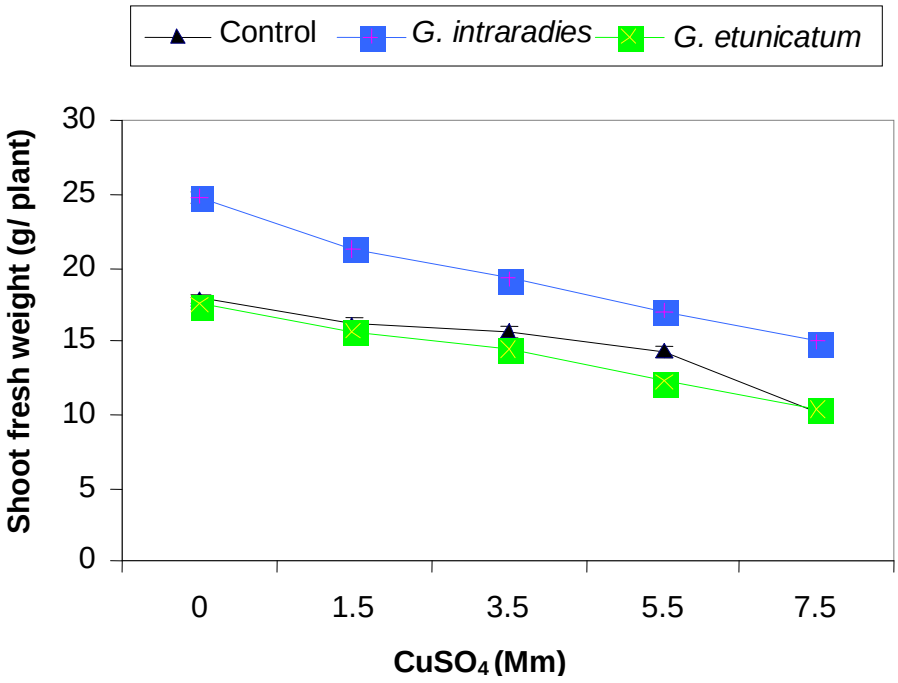

Figure 1. Effect of $CuSO_4$ on shoot fresh weight of mycorrhizal and non- mycorrhizal tomato plants.

At the end of the experiment, plants were harvested and separated into roots and shoots to obtain the fresh and dry weights (dried at 70°C for 48 h). After acid digestion treatments, the photosynthetic pigments (total chlorophyll content) in leaves were determined by the spectrophotometer according to Smith and Benitez (1955). Total sugar content in shoots and roots were determined by anthrone method described by Fales (1951) and total protein content was determined by Lowry et al. (1951).

Enzyme analyses and root colonization percentage

All samples of plants were prepared for enzyme analyses by homogenization of the fresh tissue material with a mortar and pestle and a small amount of sand in a solution (5 ml g^{-1} fresh weight) containing 50 mM KH_2PO_4 : K_2HPO_2 (pH 7.0), 10 g L^{-1} polyvinyl-polypyrrolidone (PVP), 0.2 mM EDTA and 10 ml L^{-1} Triton X-100. After the homogenate was centrifuged at 12000 × g for 20 min at 4°C, the supernatant was used for immediate determination of enzyme activities. All spectrophotometer analysis was conducted on a spectrophotometer (Beckmann, Munich, Germany).

For the extraction of antioxidative enzymes, roots were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The homogenate was centrifuged at 12,000 g for 20 min and the resulting supernatant was used for the determination of enzyme activity. The whole extraction procedure was carried out at 4°C. Guaiacol peroxidase (GUPX) activity was measured according to the method of Upadhyaya et al. (1985). Ascorbate peroxidase (APX) activity was measured according to the method of Asada and Chen (1992).

In order to determine root colonization percentage, sub-samples of fresh roots were rinsed with distilled water, cleared by 10% KOH for 15 min at 90°C, bleached in alkaline hydrogen peroxide for 20 min, acidified in 1% HCl, and stained with trypan blue for mycorrhizal colonization estimation (Kormanik and McGraw, 1982). For quantification of AMF colonization, 60 cm sections were mounted on slides (30 per slide) and colonized root tissue was evaluated as a proportion of total length of observed roots (percent root length colonized).

RESULTS

The results presented in Figures 1, 2, 3 and 4 reveal the growth responses of tomato plants grown under different Cu concentrations in the presence and the absence of AM fungi. The result indicates that AM fungi inoculations had a significant influence on shoot and root dry weight of tomato plants, where the presence of AM fungi caused a decrease in the inhibiting effects of Cu on dry weight of roots and shoots in the studied plants. According to Figures 1 and 3, roots and shoots dry weight of non-AM plants reduced in the presence of high Cu concentration in nutrient solution. The data given in Figure 5 show that chlorophyll (chl) content of AM and non –AM plants were generally reduced by increasing Cu concentration in nutrient solution. AM plants, had greater amount of chlorophyll than non-AM tomato plants. As shown in Figures 6 and 7, different Cu concentration had significant effect on total protein content in tomato plants. The protein content significantly decreased in the presence of AM fungi and reduced in the absence of fungi in non-AM plants.

The results of Figures 8 and 9 showed that soluble sugar content decreased by increasing Cu concentration in nutrient solution. However, AM plants in comparison with non-AM plants had greater amount of sugar.

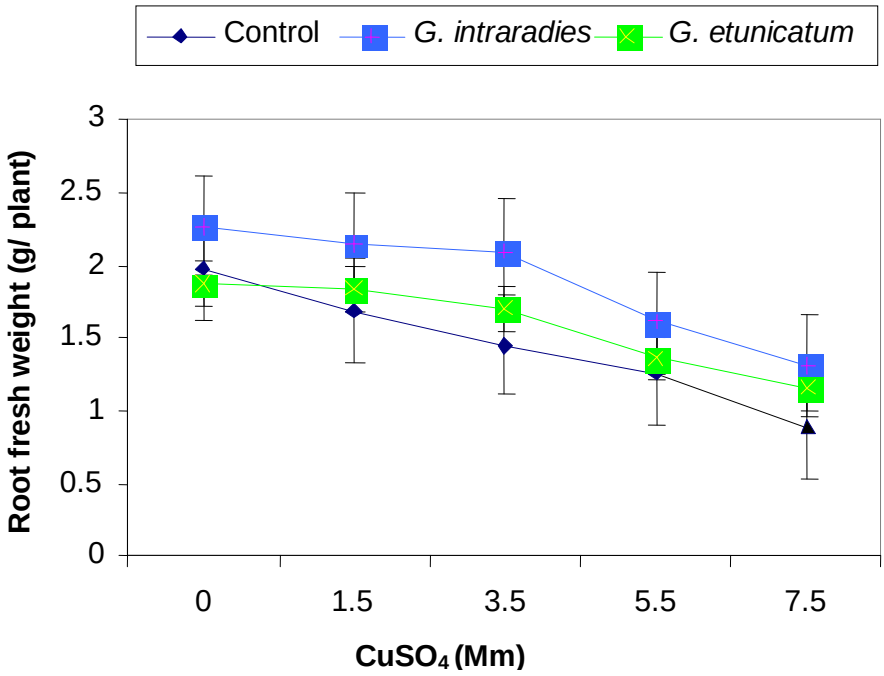

Figure 2. Effect of $CuSO_4$ on shoot dry weight of mycorrhizal and non- mycorrhizal tomato plants.

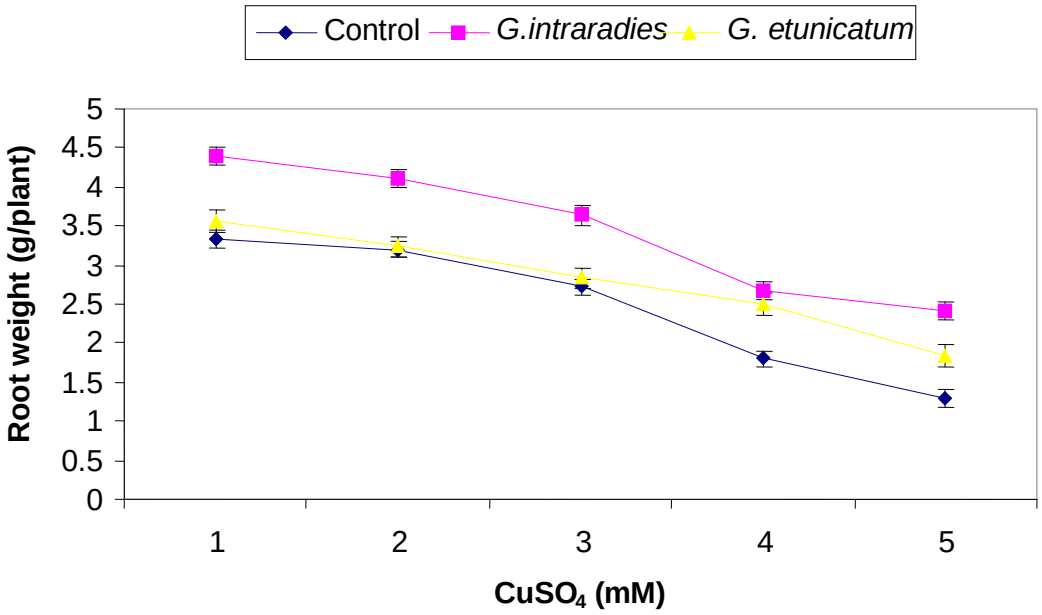

Figure 3. Effect of $CuSO_4$ on root fresh weight of mycorrhizal and non- mycorrhizal tomato plants.

The data in the present study showed that the phosphorus content of non-AM plants was generally decreased by increasing Cu concentration in nutrient solution. AM inoculation had a significant effect on phosphorus content where the value of this element obtained in the AM plants remained greater than those given by non-AM plants. According to atomic absorption spectrophotometry roots of AM plants had greater amount of Cu than shoots.

Figure 10 shows the effect of Cu treatment on the activity of APX in the shoots of mycorrhizal and non-mycorrhizal tomato plants. APX activity in mycorrhizal

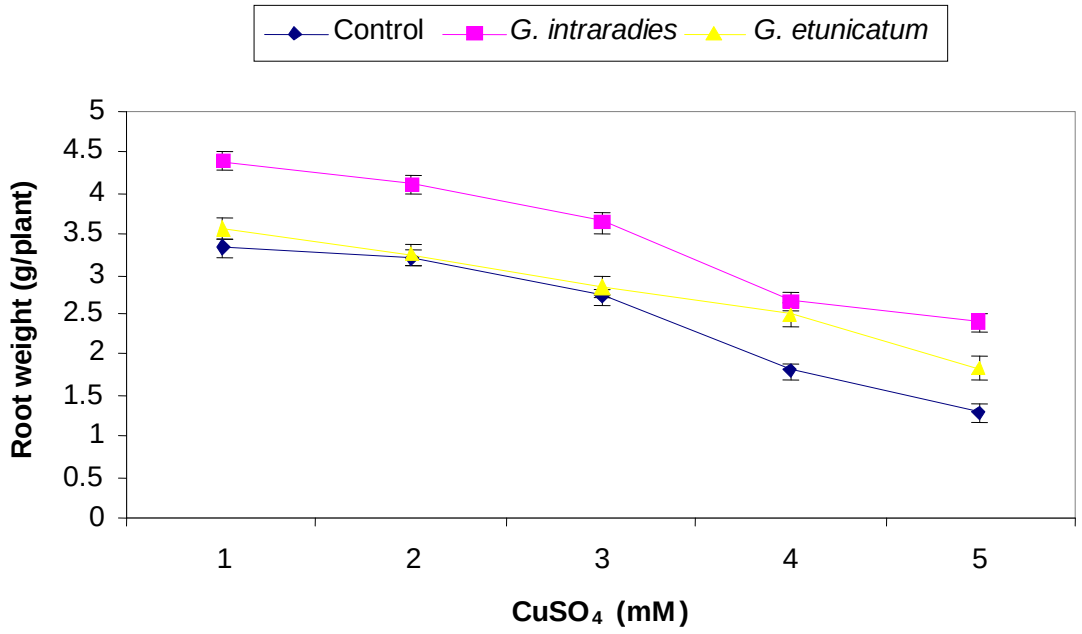

Figure 4. Effect of $CuSO_4$ on root dry weight of mycorrhizal and non- mycorrhizal tomato plants.

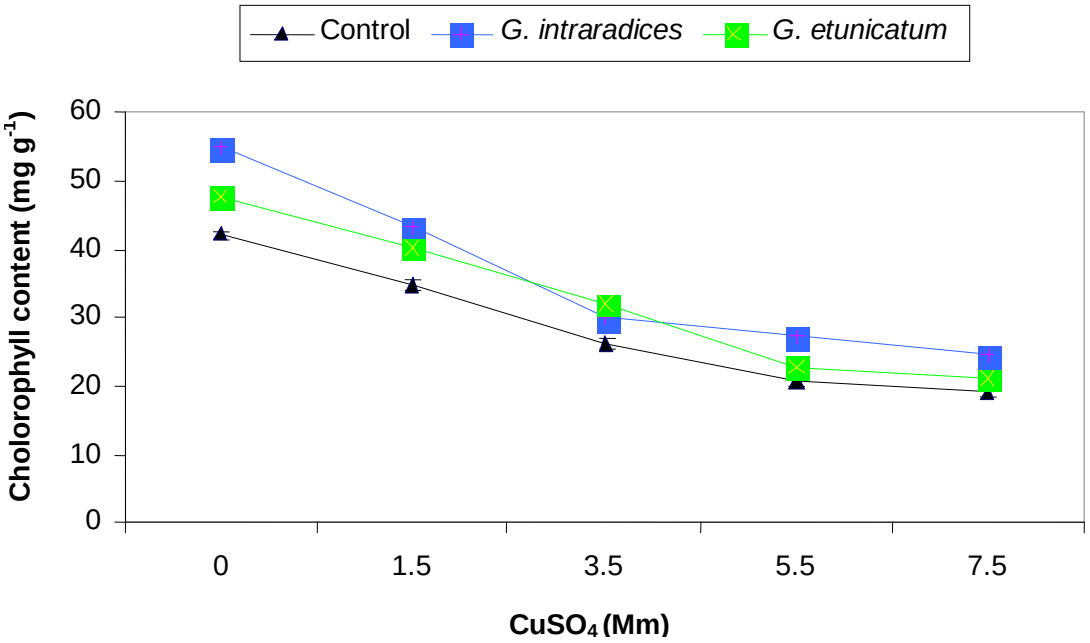

Figure 5. The effects of Cu treatment on the chlorophyll content of mycorrhizal and non- mycorrhizal tomato plants.

shoots increased significantly ($p< 0.05$) by increase in Cu concentration, but there was no significant correspondent increase in the roots of these plants (Figure 11). Activity of this enzyme in non-mycorrhizal plant increased significantly ($p< 0.05$), but activity of this enzyme in roots and shoots of mycorrhizal plants were higher than non-mycorrhizal plants. Figure 12 shows that GUPX activity in mycorrhizal roots increased significantly ($p< 0.05$) by increase in Cu concentration in nutrient solution, but there was no significant correspondent increase in the roots of these plants (Figure 13). Activity of this enzyme in non-mycorrhizal plant increased significantly ($p< 0.05$), but activity of this enzyme in roots and shoots of mycorrhizal plants were higher than non-mycorrhizal

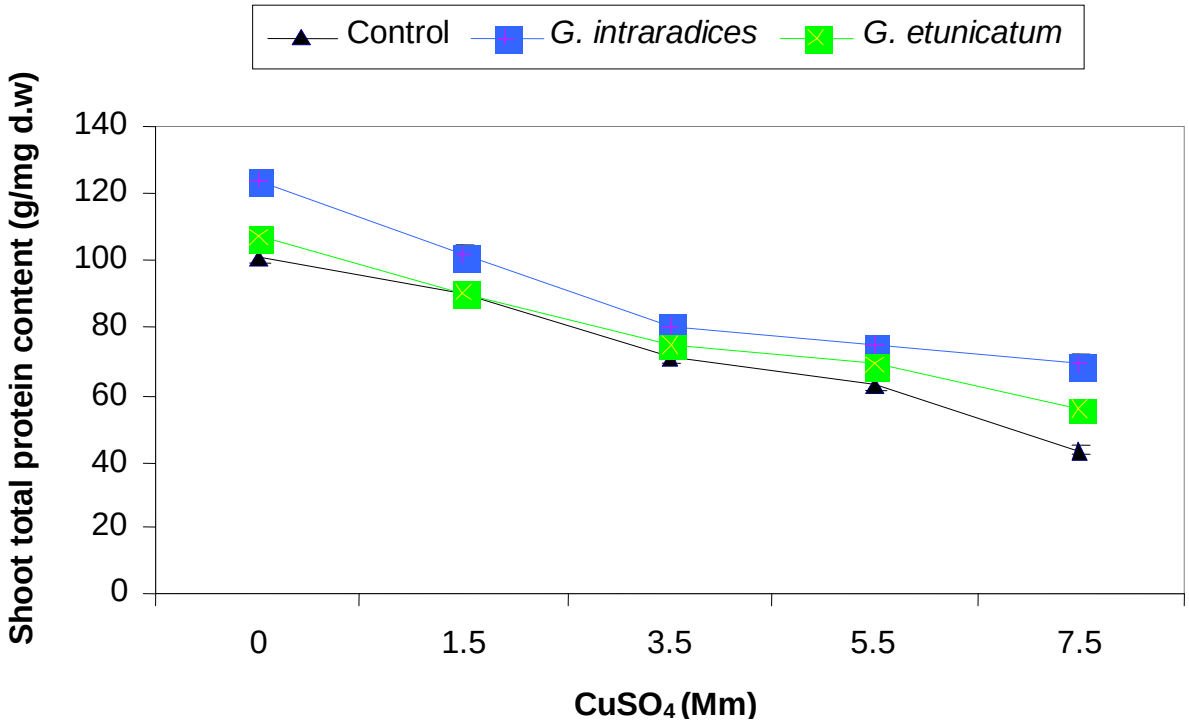

Figure 6. Effect of $CuSO_4$ on shoot protein content of mycorrhizal and non- mycorrhizal tomato plants.

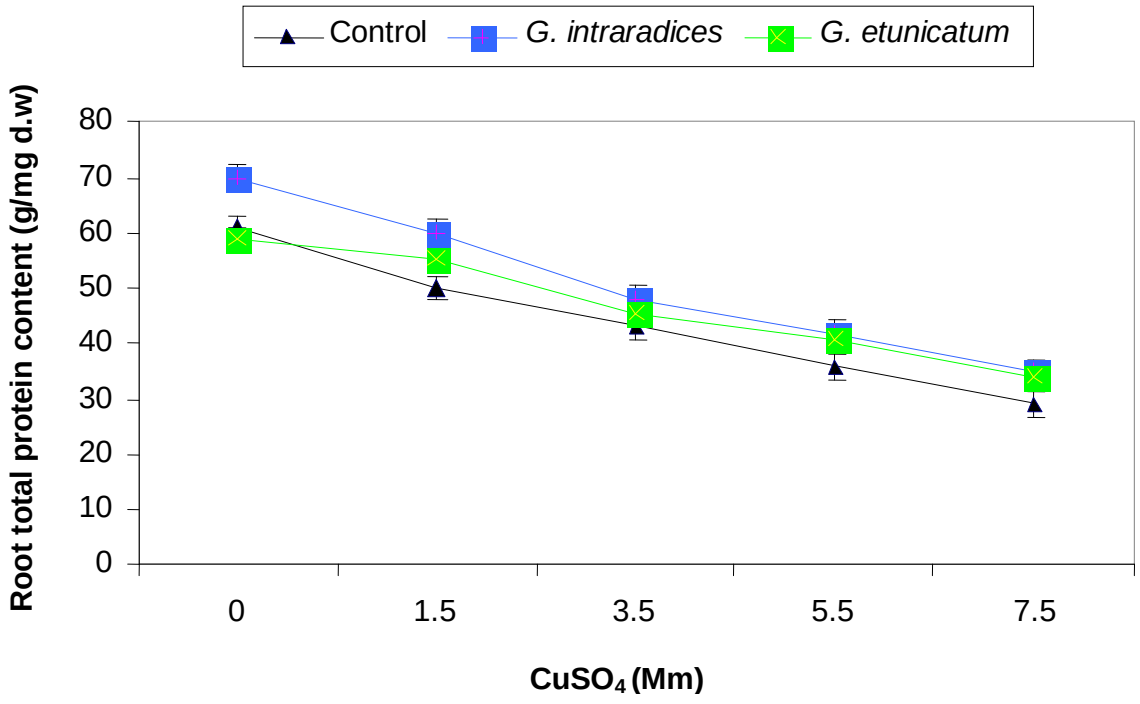

Figure 7. Effect of $CuSO_4$ on root protein content of mycorrhizal and non- mycorrhizal tomato plants.

plants. Determining of root colonization percentage by AM fungus with gridline intersect method showed a decreased in colonization by increasing in Cu concentration in nutrient solution (Figure 14).

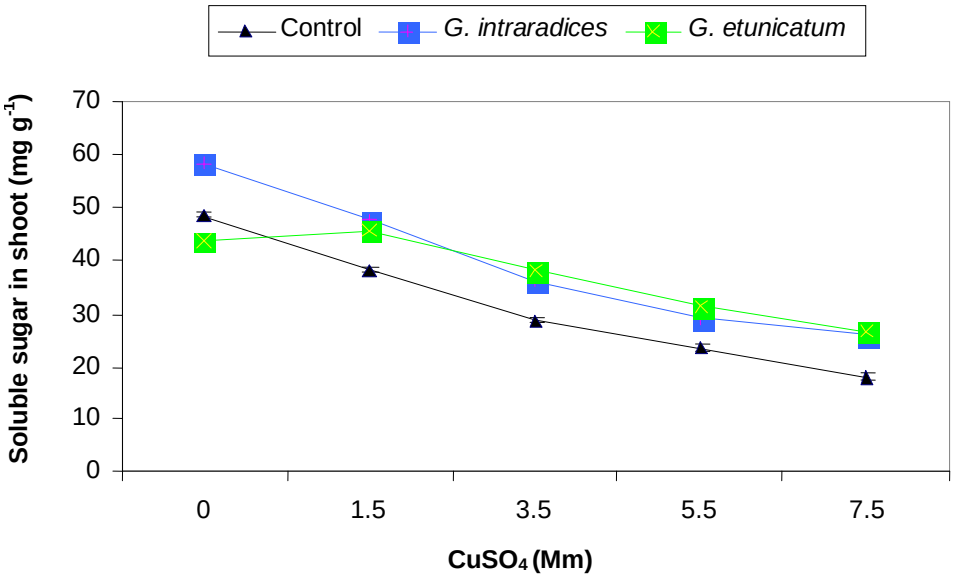

Figure 8. Effect of $CuSO_4$ on shoot soluble sugar content of mycorrhizal and non-mycorrhizal tomato plants.

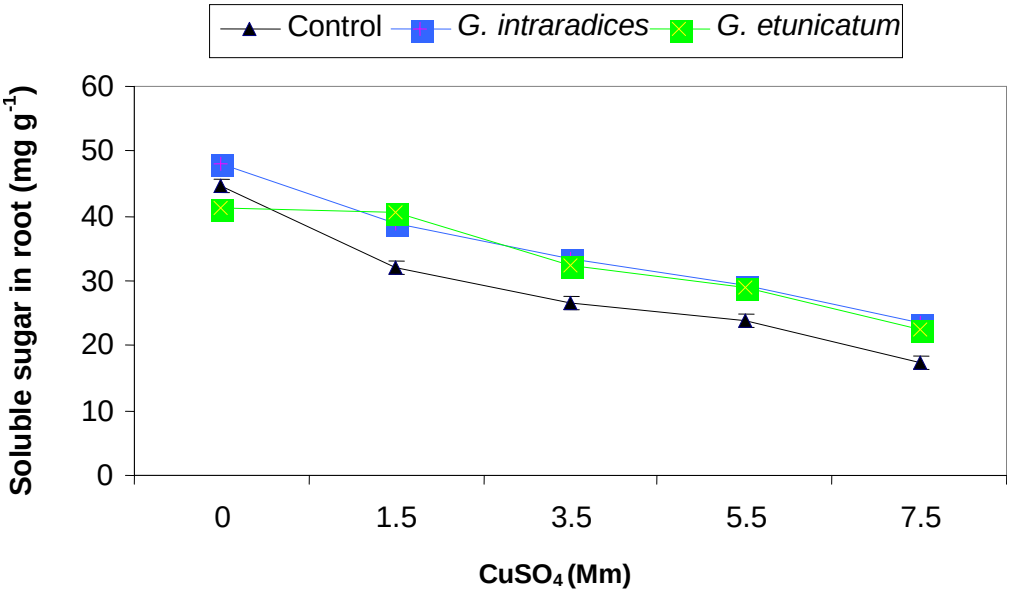

Figure 9. Effect of $CuSO_4$ on root soluble sugar content of mycorrhizal and non- mycorrhizal tomato plants.

DISCUSSION

Previous studies have reported that AM fungi decrease metal accumulation in plants, thus protecting them against heavy metal toxicity and helping them grow (Diaz, 1996; Gildon and Tinker, 1981), showed that Cu and Zn were sequestered in roots of *Calluna vulgaris* which were colonized by ericoid mycorrhizas. Reduced metal translocation from roots to shoots in the presence of AM fungi has also been shown (Joner et al., 2000; Schu¨epp et al., 1987). The accumulation of heavy metals in the fungal structures as suggested by their high heavy metal-binding capacity (Joner et al., 2000) could represent a biological barrier. In our experiment, the AM inoculum from the tomato rhizosphere was very efficient in sequestering metals in the roots.

The data in the present study showed that dry weight of roots and shoots of tomato plants would increase in the

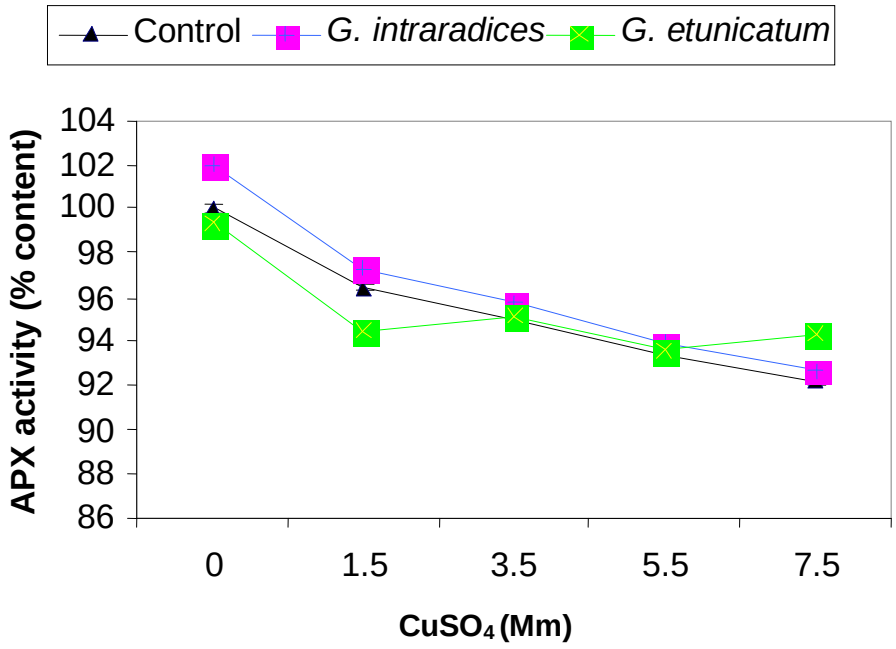

Figure 10. The effects of Cu treatment on the activity of APX in the shoots of mycorrhizal and non- mycorrhizal tomato plants treated with $CuSO_4$.

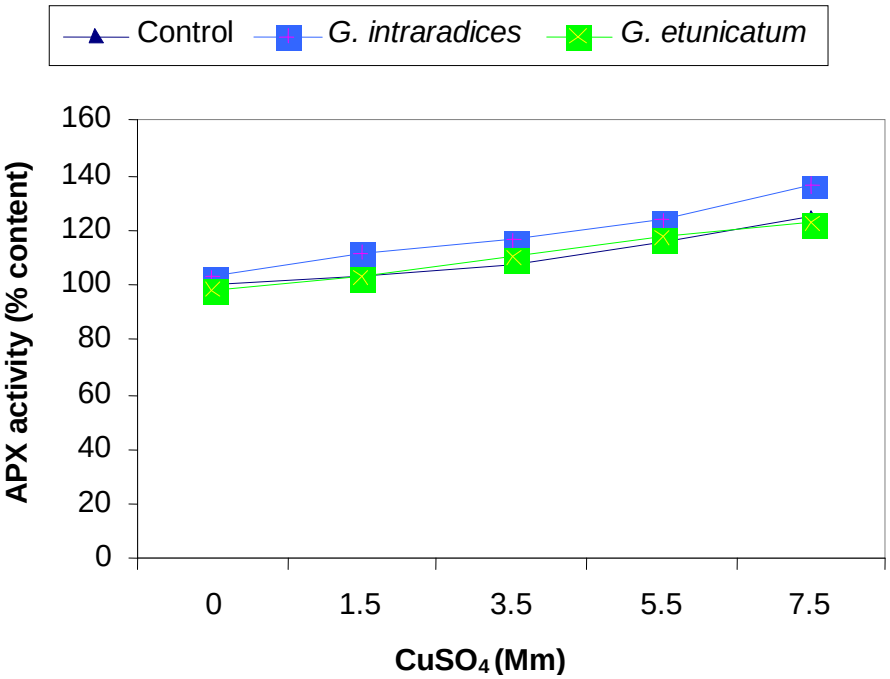

Figure 11. The effects of Cu treatment on the activity of APX in the roots of mycorrhizal and non- mycorrhizal tomato plants treated with $CuSO_4$.

presence of AM fungi. These results indicate that the known beneficial effects of mycorrhiza symbiosis appeared to be mainly due to the improvement of P uptake by mycorrhizal fungus. Ott et al. (2002) pointed out that growth inhibition in plants grown under high levels of copper, was due to interference of copper with phosphorous uptake by plants. They also reported that the application of vesicular abuscular mycorrhiza (VAM)

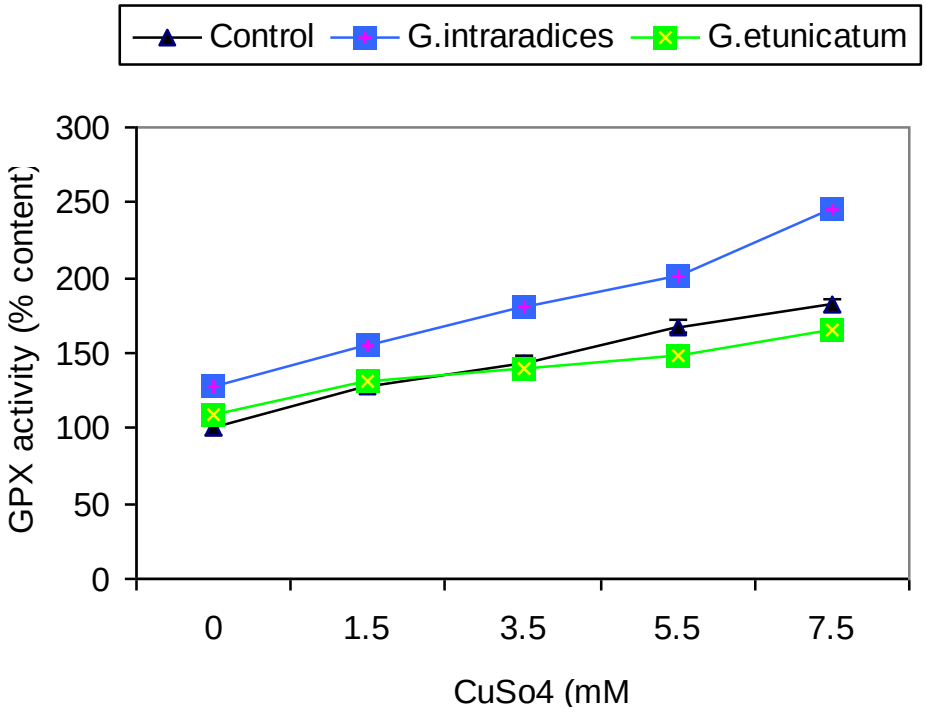

Figure 12. The effects of Cu treatment on the activity of GPX in the shoots of mycorrhizal and non- mycorrhizal tomato plants treated with $CuSO_4$.

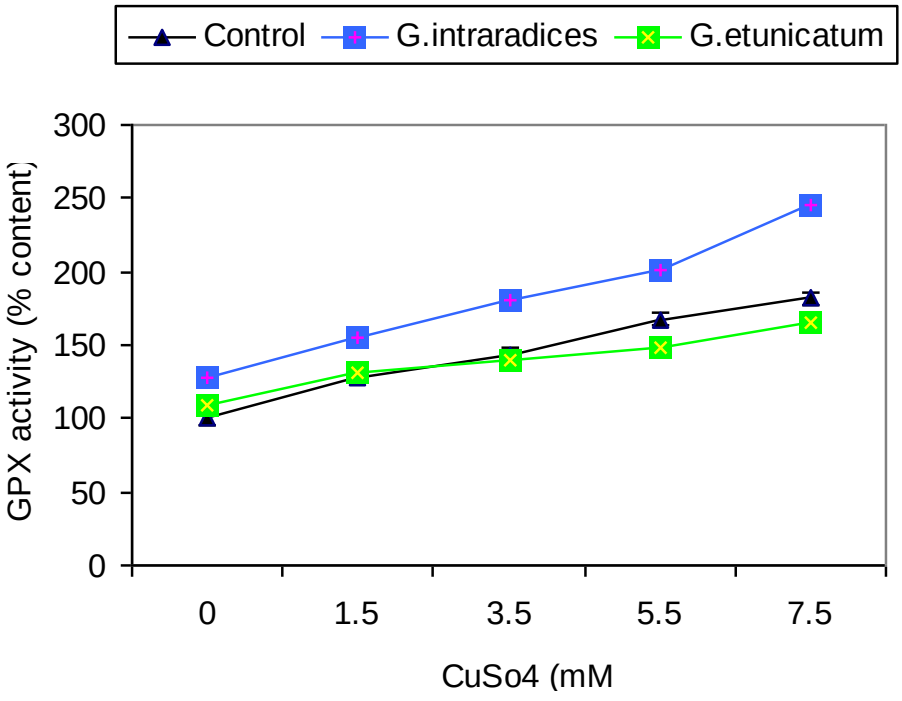

Figure 12. The effects of Cu treatment on the activity of GPX in the shoots of mycorrhizal and non- mycorrhizal tomato plants treated with $CuSO_4$.

fungi at contaminated sites increased plants biomass even at elevated copper in the soil.

As shown in Figure 5, chlorophyll content of AM and non-AM tomato plants decreased in the presence of high

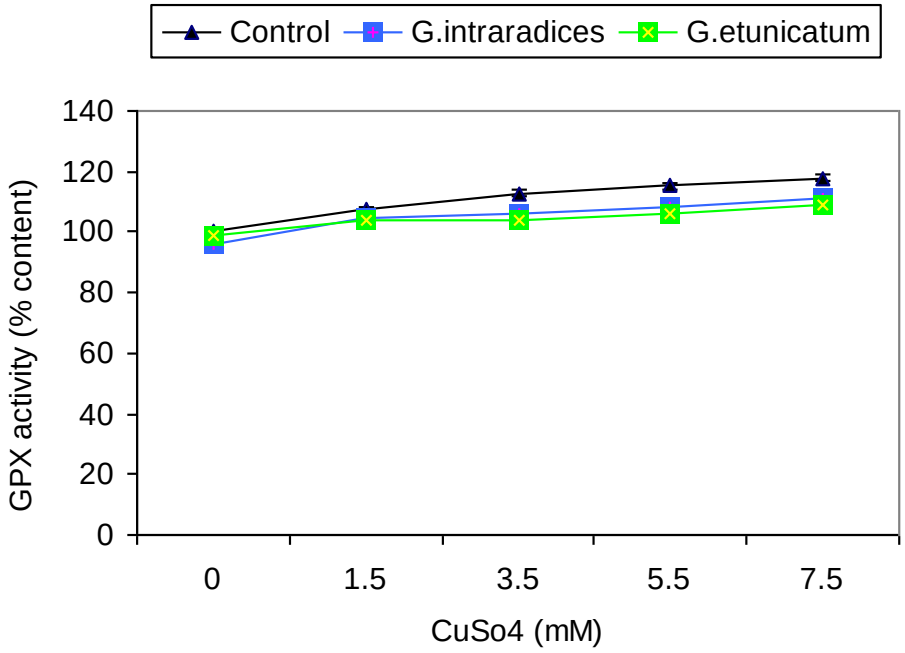

Figure 13. The effects of Cu treatment on the activity of GPX in the roots of mycorrhizal and non- mycorrhizal tomato plants treated with $CuSO_4$.

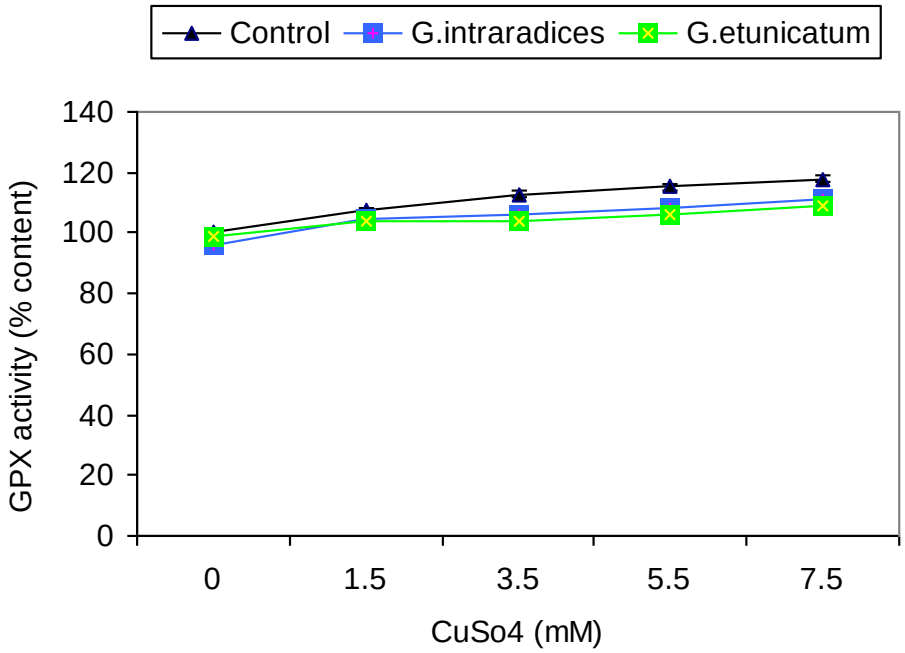

Figure 13. The effects of Cu treatment on the activity of GPX in the roots of mycorrhizal and non- mycorrhizal tomato plants treated with $CuSO_4$.

Cu concentrations. Copper induced inhibition of Fe translocation from root to tops needed for chlorophyll synthesis and therefore causes chlorosis in plant. Joner et al. (2000) reported that Cu toxicity appear to be due to Fe deficiency. White et al. (1976) observed that increased levels of Cu in soil greatly increased translocation of Mn to tops which indicates the appearance of chlorosis. They hypothesized that Cu, Zn and Mn interfere with Fe utilization in the leaves for chlorophyll synthesis. In the present study, AM plants

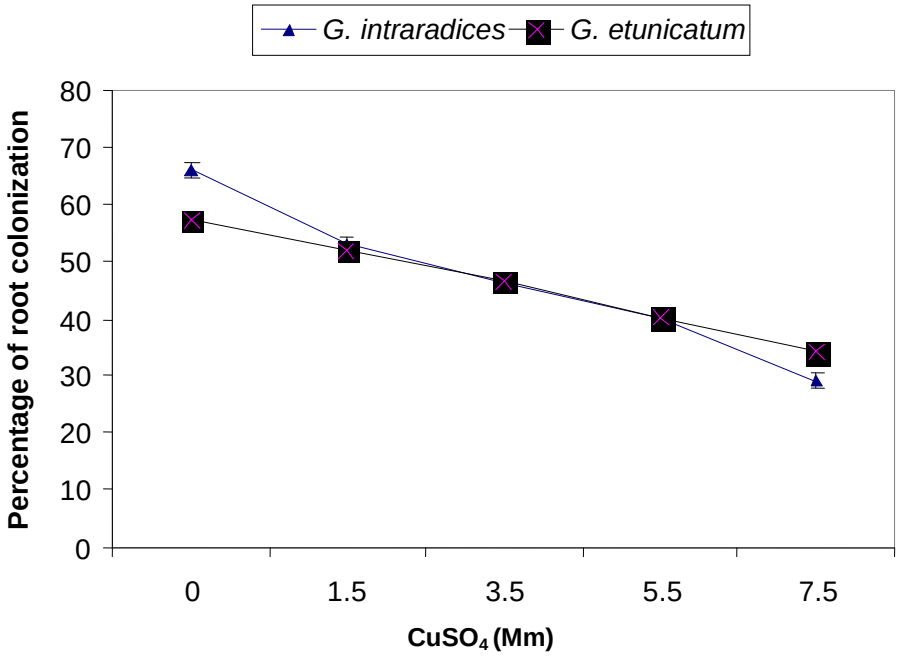

Figure 14. The effects of Cu treatment on the percentage of root colonization with *Glomus etunicatum* in tomato plants.

possess greater amount of chlorophyll in comparison with non-AM plant, which indicate to alteration of Cu translocation pattern from root to shoot (Ott et al., 2002). An inhibition of Cu translocation to shoots was also reported in mycorrhizal maize seedlings (Khan et al., 2000).

The data of the present study in Figures 5 and 6 showed that total protein content increased in the presence of mycorrhizae fungi but it reduced in the absence of fungi. Based on these results, the mechanisms related to physiological interactions between AM fungus and tomato plants involve increase in protein synthesis as well as induction of antioxidant enzymes to avoid heavy metal-mediated oxidative stress. This finding supports results from some studies reporting the role of "stress proteins" such as phytochelatins and metallothioneines (Gildon and Tinker, 1983; Rauser, 1999), as possible mechanisms for protection against high toxic heavy metals (Burleigh et al., 2003; Ott et al., 2002; Tong et al., 2004). In non-mycorrhizal tomato plants, reduction in total protein content may be due to the toxic effects of copper on cellular metabolism and protein synthesis.

According to Figures 8 and 9, reduction in soluble sugar in both AM and non-AM plants may be due to a decrease in chlorophyll content and the abnormal structure of chloroplasts, which leads to low photosynthesis efficiency. Mycorhizal plants alleviate the severe effect of Cu by changing the translocation of copper and sequestering it in their hypha; so the toxic effects of Cu on photosynthesis and carbohydrate metabolism might decrease. Reduced amount of phosphorous which observed in non-AM plants may be due to interference of copper with phosphorous uptake by tomato plants. The great amount of P in mycorrhizal plants emphasizes the enhancement of P uptake from the soil and its translocation to plants by the extrardial mycelium of AM fungi (Rufyikiri et al., 2004; Ryan and Angus, 2003; Vogel-Mikus et al., 2005). The protective mechanisms adapted by plants to scavenge free radicals and peroxides include several antioxidative enzymes such as SOD, CAT and POD. The antioxidative enzymes are important components in preventing the oxidative stress in plants as is based on the fact that the activity of one or more of these enzymes is generally increased in plants when exposed to stressful conditions (Vogel-Mikus et al., 2005). Figures 10 to 14 indicates antioxidant anzyme (APX and GUPX) activity of AM tomato plants which show significant higher values in polluted soil than in non-polluted one. Based on these results, the mechanism related to physiological interactions between the AM fungus and tomato plants involve increased protein synthesis as well as induction of antioxidant enzymes to avoid heavy metal-mediated oxidative stress. If so, the author attributed the reduced heavy metal toxicity effects in AM tomato plants to antioxidative protection through detoxification of heavy metals, chelation through metal-binding proteins (peptides) and dilution through increased plant growth induced by AM fungi. This finding supports the results from some dissipated studies that the role of protein, antioxidative enzyme system as well as improved growth as possible

mechanisms for plant protection against high accumulated toxic heavy metals in the shoots (Burleigh et al., 2003; Ott et al., 2002; Tong et al., 2004). This finding is in line with the results of Leyval et al. (1997) and Vogel- Mikus et al. (2005), who reported that sensitivity of AM symbionts to heavy metal contaminated soil expressed as a reduction in spore germination, hyphal growth or root colonization had been proved previously in a number of studies. Another interesting result in figure 2 was that the presence of AM fungi inoculations would increase the metal tolerance index of tomato plants compared with non-AM plants that grew in heavy metal polluted soil. This result emphasizes that AM fungi could be potentially effective in protecting plants exposed to high levels of heavy metal. The AM fungi ability to alleviate heavy metal stress of plants grown in heavy metal contaminated soil was previously proved by Rufyikiri et al. (2004) and Burleigh et al. (2003).

Estimation of root length colonization by gridline intersect method, resulted in 50% decrease between controls (0 mM) and 7.5 mM and showed that by increase in Cu concentration, colonization percentage decrease significantly. These results clearly indicate the beneficial effect of AM fungi in the protection of plants and alleviation of the toxic effect of copper.

Therefore, additional researches are needed to explore the behavior of AM fungi in various plant species and families for plant protection under heavy metal stress.

REFERENCES

Arduini I, Godbold DL, Onnis A (1995). Influence of copper on root growth and morphology of Pinus pinea L. and Pinus pinaster Ait. seedlings. Tree Physiology, 15: 411-415.

Asada K, Chen GX (1992). Ascorbate peroxidase- a hydrogen peroxide scavenging enzyme in plants. Physiol. Plant. 85: 235-241.

Burleigh SH, Kristensen BK, Bechmann IE (2003). A plasma membrane zinc transporter from Medicago truncatula is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol. Biol. pp. 1077-1088(12)

Choi J, Pak C, Lee CW (1996). Micronutrient toxicity in French marigold. J. Plant Nutri. 19: 901-916.

Diaz G, Azcón-Aguilar C, Honrubia M (1996). Influence of arbuscular mycorrhiza on heavy metal (Zn and Pb) uptake and growth of Lygedum spartum and Anthyllis cytisoides. Plant Soil,180: 241-249.

El-Kherbawy M, Angle JS, Heggo A, Chaney RL (1989). Soil pH, rhizobia, and vesicular–arbuscular mycorrhizae inoculation: effects on growth and heavy metal uptake of alfalfa (Medicago sativa L.). Biol Fert Soils, 8: 61-5.

Entry JA, Rygiewicz PT, Watrud LS, Donnelly PK (2002). Influence of adverse soil conditions on the formation and function of Arbuscular mycorrhizas. Adv. Environ. Res. 7 (1): 123-138.

Fales FW (1951). The assimilation and degradation of carbohydrates by yeast cells. J Biol Chem. 193: 113-124.

Galli U, Schu¨epp H, Brunold C (1994) Heavy metal binding by mycorrhizal fungi. Physiol Plantarum,92: 364-8.

Gildon A, Tinker PB (1981). A heavy metal-tolerant strain of a mycorrhizal fungus. Trans Br Mycol Soc 77: 648-649.

Gildon A, Tinker PB (1983). Interactions of vesicular–arbuscular mycorrhizal infections and heavy metals in plants, II. the effects of infection on uptake of copper. New Phytol, 95: 263-8.

Jakobsen I, Abbott LK, Robson AD (1992). External hyphae of vesicular–arbuscular mycorrhizal fungi associated with Trifolium subterraneum L, 1: spread of hyphae and phosphorus inflow into roots. New Phytol. 371-80.

Johansen A, Jakobsen I, Jensen ES (1992). Hyphal transport of 15N-labelled nitrogen by a vesicular–arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol, 122: 281-8.

Joner EJ, Briones R and Leyval C (2000). Metal binding capacity of arbuscular mycorrhizal mycelium. Plant Soil, 226: 227-234.

Kaldorf M, Kuhn AJ, Schroder WH, Hildebrandt U, Bothe H (1999). Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. J Plant Physiol, 154: 718-28.

Khan AG, Chaudhry TM, Khoo CS, Hayes WJ (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere, 41: 197-207.

Kormanik PP, McGraw AC (1982). Quantification of VA mycorrhizae in plant roots. In: Schenk NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St Paul, Minn, pp. 37-45.

Kothari SK, Marschner H, Romheld V(1991). Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant Soil, 131:177-85

Lee CW, Jackson MB, Duysen ME, Freeman TP, Self JR (1996). Induced micronutrient toxicity in 'Towndown' Kentucky bluegrass. Crop Science. 36, 705-712

Leyval C, Turnau K, Haselwandter K (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza, 3: 139-53.

Li XL, Christie P (2001). Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. Chemosphere, 42: 201-207.

Lowry OJ, Rosebrough AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Malcova R, Vosatka M, Gryndler M (2003). Effects of inoculation with Glomus intraradices on lead uptake by Zea mays L. and Agrostis capillaris L. Appl. Soil Ecol. 23(1): 255 - 267.

Minnich MM, McBride MB, Chaney RL (1987). Copper activity in soil solution. II. Relation to copper accumulation in young snapbeans. Soil Sci. Soc. Am. J. 51: 573-578.

O'Sullivan JN, Asher CJ, Blamey FPC (1997). 'Nutrient Disorders of Sweet Potato. ACIAR Monograph No. 48.' Australian Centre for International Agricultural Research, Canberra

Ott T, Fritz E, Polle A, Schutzendubel A (2002). Characterization of antioxidative systems in the ectomycorrhiza -building basidiomycete Paxillus involutus (Bartsch) Fr. and its reaction to cadmium. FEMS Microbiol. Ecol. 42: 359-366.

Patterson W, Olson, JJ (1983). Effects of heavy metals on radicle growth of selected woody species germinated on filter paper, mineral and organic soil substrates. Canadian J. Forest Res.13: 233-238.

Rauser WE (1999). Structure and function of metal chelators produced by plants—the case for organic acids, amino acids, phytin and metallothioneins. Cell Biochem Biophy. 31: 19-48.

Rufyikiri G, Huysmans L, Wannijn J, Van Hees M, Leyval C, Jakobsen I (2004). Arbuscular mycorrhizal fungi can decrease the uptake of uranium by subterranean clover grown at high levels of uranium in soil. Environ. Pollut. 130: 427- 436.

Ryan MH, Angus JF (2003). Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in Puptake or yield.Plant Soil, 250 (2): 225-239.

Schu¨epp H, Dehn B, Sticher H (1987). Interaktionen zwischen VAMykorrhizen und Schwermetallbelastungen. Angew Bot. 61:85-96.

Smith JHC, Benitez A (1955).Chlorophylls analysis in plant materials. In: Paeh K, Tracey MV eds. Modern Methods.Plant Anal. 4:159.

Taylor GJ, Foy CD (1985). Differential uptake and toxicity of ionic and chelated copper in Triticum aestivum. Canadian J.Bot. 63: 1271-1275.

Tong Y, Kneer R, Zhu Y (2004). Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. Trends Plant Sci. 9: 7-9.

Turnau K, Mesjasz-Przybylowicz J (2003). Arbuscular mycorrhizal of Berkheya codii and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. Mycorrhiza, 13:185-190.

Vogel-Mikus K, Drobne D, Regvar M (2005). Zn, Cd and Pb

accumulation and arbuscular mycorrhizal colonisation of pennycress Thlaspi praecox Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. Environ. Pollut. 133: 233-242.
Upadhyaya A, Sankla D, Davis TD, Sankhla N, Smith BN (1985). Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senecing soybean leaves. Physiol. Plant. 127: 453-461.
Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995). Arbuscular mycorrhizal contribution to heavy metal uptake by maize (Zea mays L.) in pot culture with contaminated soil. Mycorrhiza, 5: 245-51.
White RE (1976). Studies on the mineral ion absorption by plants. III. The interaction of aluminium phosphate and pH on the growth of Medicago sativa, Plant Soil, 46 :195-208.
Yau PY, Loh CF, Azmil IAR (1991). Copper toxicity of clove [Syzygium aromaticum (L.) Merr. and Perry] seedlings. MARDI Res. J. 19: 49-53.
Zhu B, Alva AK (1993). Effect of pH on growth and uptake of copper by swingle citrumelo seedlings. J.Plant Nutri. 16: 1837-1845.